IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Ji Sook Park, et al.
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For: PROCESS FOR PURIFYING INTERFERON BETA
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Art Unit: 1646
Examiner Bruce D. Hissong, Ph.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Ahn, Jee Won declares and says:

1. I am an inventor of the above-referenced application.

2. I received a B.S. in Food and Microbial technology from Seoul Women's University in 2000 and a M.S. in Department of Biotechnology from Yonsei University in 2002. Since 2002, I have been employed by CJ Corporation, 511 Deokpyeng-ri, Majang-myeon, Icheon-si, Gyeonggi-do, Republic of Korea and have been engaged in research in the field of biopharmaceutics.

3. Carter discloses that propylene glycol (PG) is more desirable than ethylene glycol (EG) in eluting interferon beta from affinity ligand; however, it does not disclose that PG increases the purity of interferon when used as a washing solution.

In these Experimental results, in order to identify the effect of PG and EG on the purity of interferon beta, interferon beta was isolated according to a method described in Example 1 of the present application and a method described in Example 1 of the present application except that PG is replaced with EG, and the purity of interferon beta in the finally eluted solution was analyzed by using HPLC.

Further, the purity of interferon beta in a solution obtained after each washing step and elution step was analyzed by using HPLC.

4. Effects of propylene glycol on the purity of interferon beta

350 ml of Blue-Sepharose 6 (Amersham biosciences, Sweden) was packed in a XK-50 column (Amersham biosciences, Sweden) to make an affinity chromatography column. A 20 mM sodium phosphate buffer solution containing 1mM EDTA was allowed to sufficiently flow through the column to equilibrate the column. Then, 25 L of a Chinese hamster ovary (CHO) cell serum-free culture containing interferon beta was allowed to flow through the column at a flow rate of 5-10 ml/min and then the column was washed with

about 3 column volumes (CV) of an equilibration buffer solution.

About 3 CV of a 20 mM sodium phosphate buffer solution (pH 7.2) containing 50 % propylene glycol was allowed to flow through the column at a flow rate of 5 ml/min to remove impurity proteins, followed by washing with about 3 CV of an equilibration buffer solution. Then, about 3 CV of 20 mM sodium phosphate buffer solution (pH 7.2) containing 2M NaCl was allowed to flow through the column at a flow rate of 5 ml/min to remove impurity proteins. Finally, about 3 CV of 20 mM sodium phosphate buffer solution (pH 7.2) containing 2M NaCl and 20% propylene glycol was allowed to flow through the column at a flow rate of 5 ml/min to remove impurity proteins.

About 3 CV of an elution buffer solution (20 mM sodium phosphate buffer solution containing 2M NaCl and 50% propylene glycol, pH 7.2) was allowed to flow through the column at a flow rate of 5 ml/min to thereby recover an interferon beta-containing solution. The purity of the eluted solution thus recovered was measured using C4 HPLC analysis chromatography and the result is shown in FIG. 1.

As a control, affinity chromatography was performed according to the above-described manner except that propylene glycol was replaced with ethylene glycol. The purity of the resultant eluted solution was measured using C4 HPLC analysis

chromatography and the result is shown in FIG. 2.

As shown in FIGS. 1 and 2, interferon beta was eluted at 15 minutes with a single peak in FIG. 1, while there are several peaks around the main peak in FIG. 2, indicating that various impurities are eluted together with the interferon beta in FIG. 2. Therefore, the Experimental Results clearly show that the propylene glycol can remarkably increase the purity of interferon beta in the affinity chromatography when used as a washing solution.

5. The effect of washing step on the purity of interferon beta

The purity of the interferon beta in a solution recovered after each washing step and elution step according to the isolation method described in section 1 was measured using C4 HPLC analysis chromatography and the result is shown in FIGS. 3A-3D.

FIG. 3A shows the purity of the interferon beta in a solution recovered after the washing step with washing buffer A: 20 mM sodium phosphate / 50% propylene glycol (pH 7.2).

FIG. 3B shows the purity of the interferon beta in a solution recovered after the washing step with washing buffer C: 20 mM sodium phosphate / 2 M NaCl (pH 7.2).

FIG. 3C shows the purity of the interferon beta in a solution recovered after the

washing step with washing buffer B: 20 mM sodium phosphate / 2 M NaCl / 20% PG (pH 7.2).

FIG. 3D shows the purity of the interferon beta in a solution recovered after the eluting step with eluting buffer: 20 mM sodium phosphate / 2 M NaCl / 50% PG (pH 7.2)

According to FIG. 3A, washing with washing solution A eluted impurities around 17 minutes and substantially did not elute interferon beta around 15 minutes. This is an unexpected result considering that 50% propylene glycol is used an elution solution (see Example 1, 2nd paragraph of Carter).

According to FIG. 3B, washing with washing solution C eluted impurities around 11 minutes and substantially did not elute interferon beta around 15 minutes.

According to FIG. 3C, washing with washing solution B eluted various impurities around 15 minutes and a small amount of interferon beta around 15 minutes.

According to FIG. 3D, eluting with elution solution eluted interferon beta around 15 minutes with a very high purity.

6. Conclusion

According to the above experimental results, the purity of the interferon beta after affinity chromatography remarkably increased by introducing three washing steps: washing

with washing solution A, washing with washing solution C, and washing with washing solution B.

Date: 2009. 12. 21 Ahn, JeeWon

Inventor name (signature)

Fig. 1

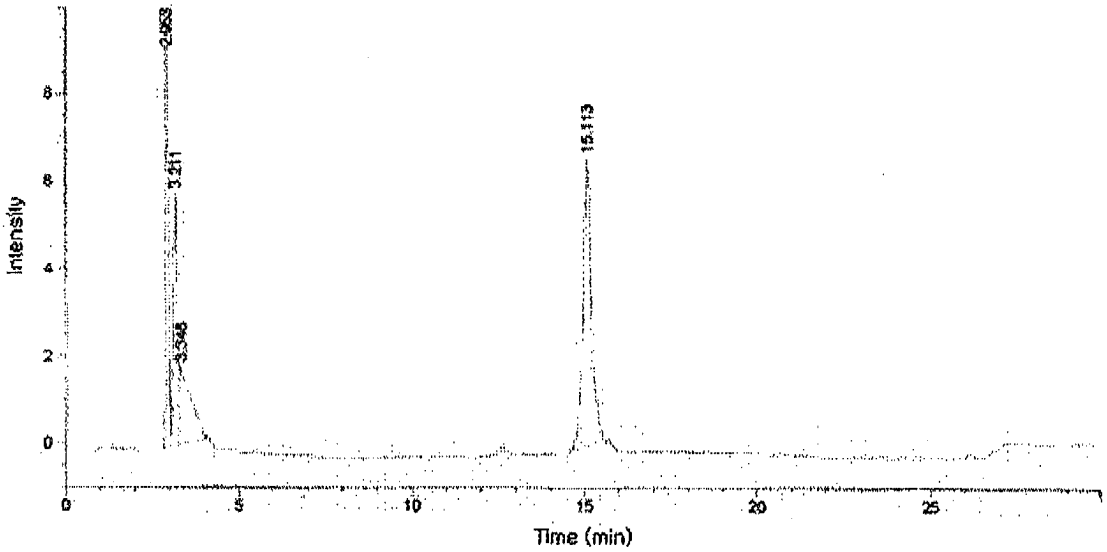


Fig 2.

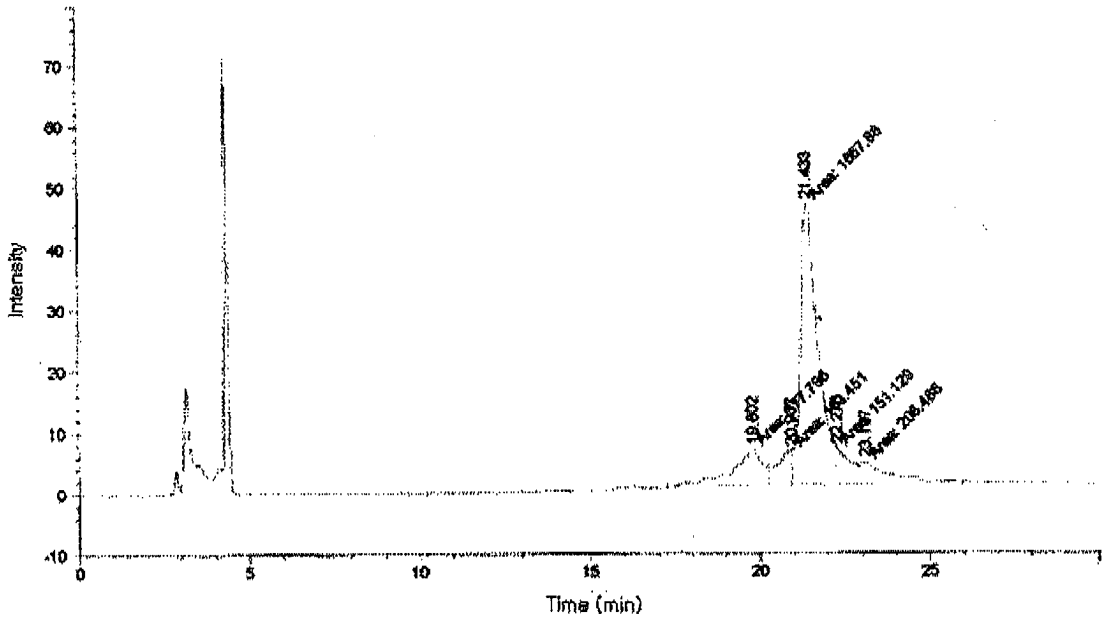


Fig 3A.

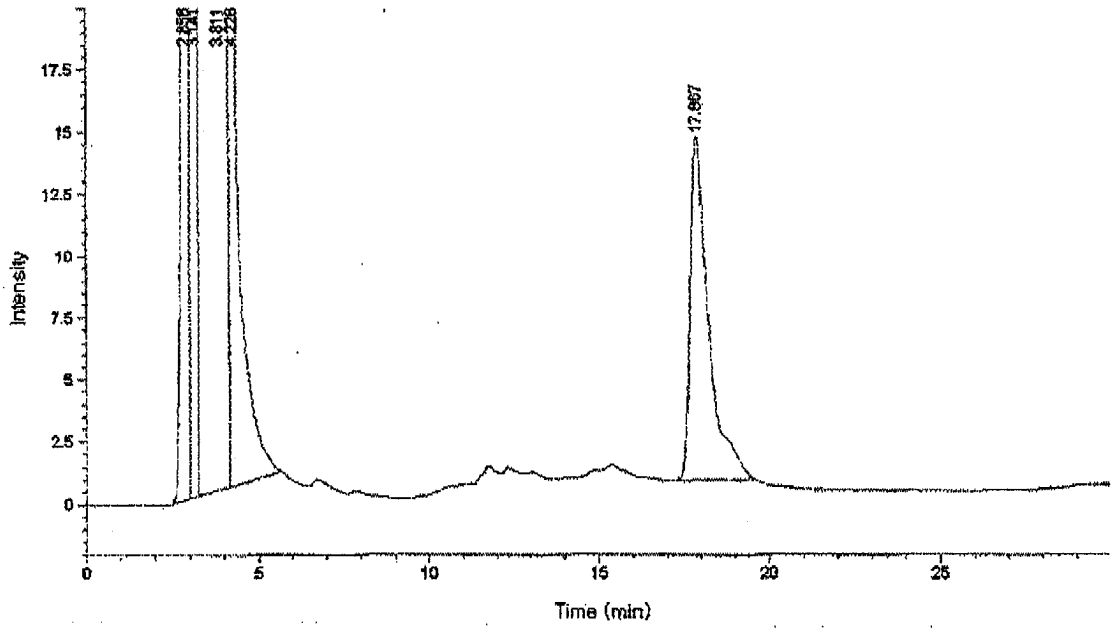


Fig 3B.

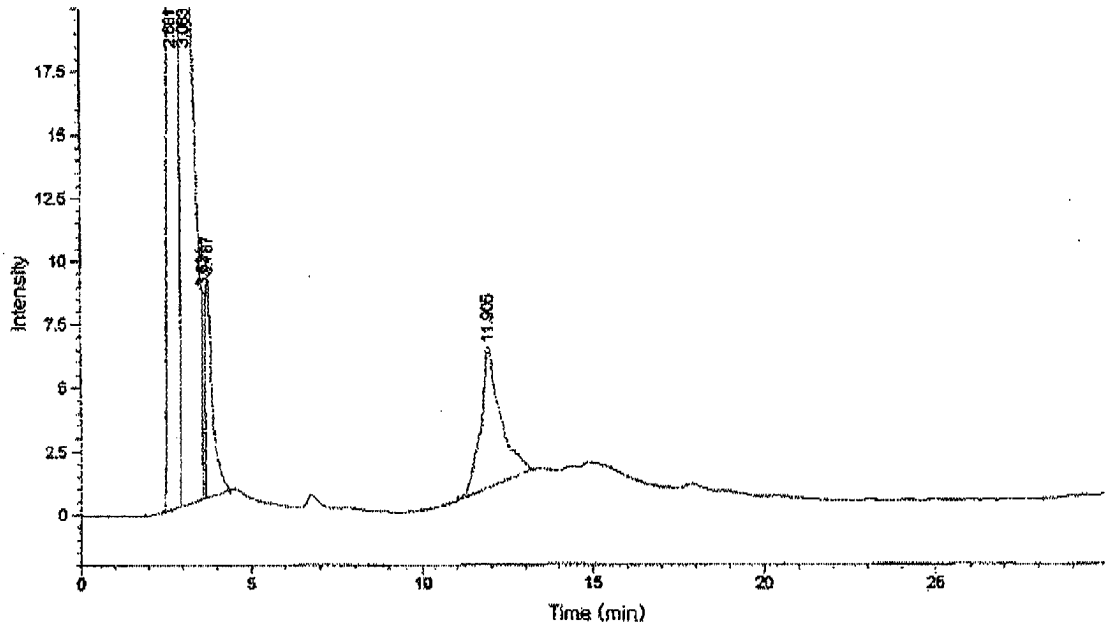
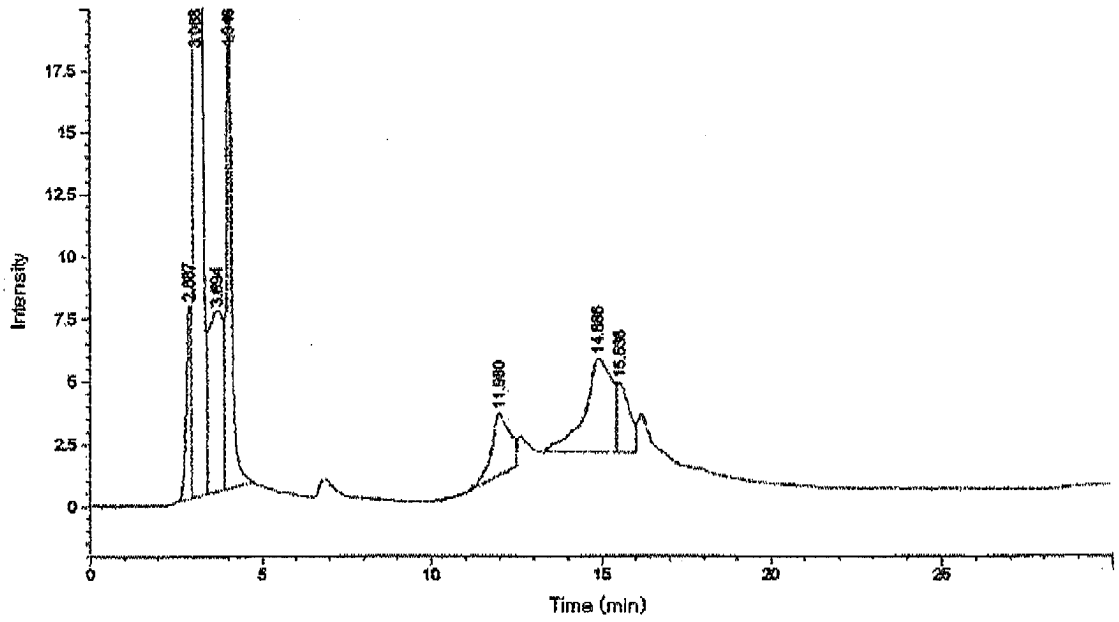


Fig 3C.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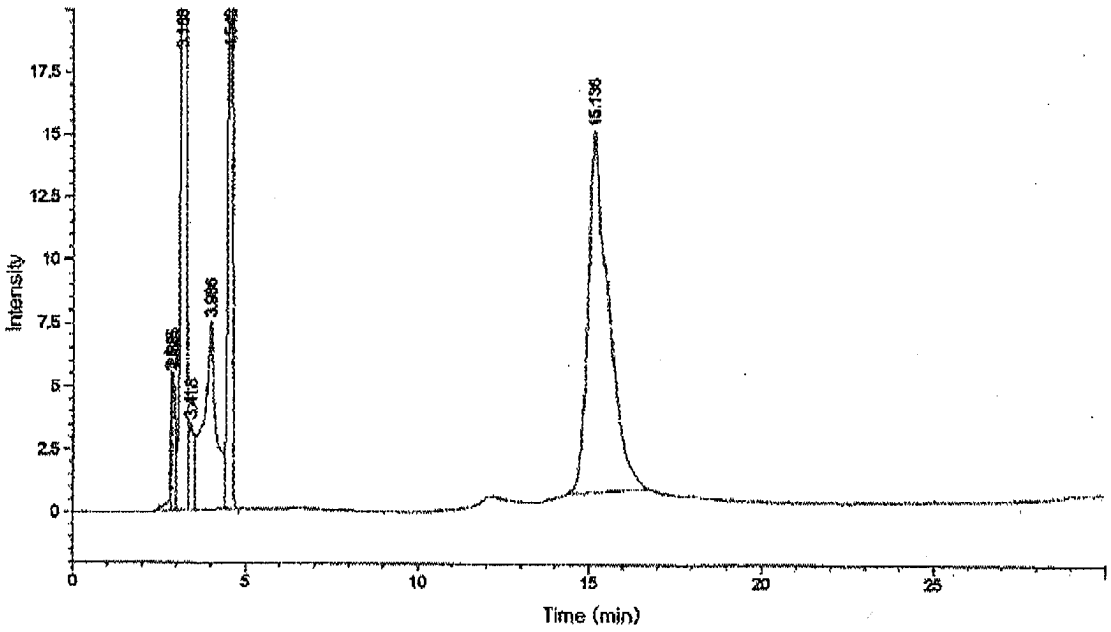
5
10
15
20
25
Time (min)

Fig 3D.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Time (min)